Safety, pharmacokinetics and antimalarial activity of the novel triaminopyrimidine ZY-19489: a first-in-human, randomised, placebo-controlled, double-blind, single ascending dose study, a pilot food effect study, and a volunteer infection study

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ABSTRACT

Background

New antimalarials with novel mechanisms of action are needed to combat the emergence of drug resistance. Triaminopyrimidines comprise a novel antimalarial class identified in a high-throughput screen against asexual blood-stage *Plasmodium falciparum*. This first-in-human study characterised the safety, pharmacokinetics and antimalarial activity of the triaminopyrimidine ZY-19489 in healthy volunteers.

Methods

A three-part clinical trial was conducted in Brisbane, Australia. Part 1 was a double-masked, randomised, placebo-controlled, single ascending dose study. Part 2 was an open-label, randomised, two-period cross over, pilot food effect study. Part 3 was an open-label, randomised, volunteer infection study using the *Plasmodium falciparum* induced blood-stage malaria model. Randomisation schedules were generated electronically. Healthy adults aged 18-55 years were eligible for inclusion. The primary outcome was the incidence, severity and relationship to ZY-19489 of adverse events (AEs). Secondary outcomes were pharmacokinetic and pharmacodynamic (parasite clearance) parameters. Trial registration (Australian New Zealand Clinical Trials Registry): ACTRN12619000127101 (part 1); ACTRN12619001466134 (part 2); ACTRN12619001215112 (part 3).

Findings

Forty-eight participants enrolled in part 1 (n=8 per cohort for 25-1500 mg cohorts, randomised 6 ZY-19489: 2 placebo), 8 in part 2 (randomised 4 fasted-fed sequence: 4 fed-fasted sequence, all dosed with 300 mg in each period), and 15 in part 3 (200 mg, n=5; 300 mg n=8; 900 mg, n=2). In part 1, the incidence of drug-related AEs was higher in the 1500 mg dose group (6/6 participants) compared to lower dose groups or placebo (1/6 for 25 mg; 2/6 for 75 mg and 450 mg; 3/6 for 150 mg; 4/6 for 900 mg; 4/12 participants for placebo),

due to the occurrence of mild gastrointestinal symptoms. Maximum plasma concentrations occurred 5-9 h post-dosing and the elimination half-life was 50-97 h across the dose range in part 1. In part 2, dosing in the fed state delayed absorption (maximum plasma concentration occurred 12·0 h fed [range 7·5-16·0] vs 6·0 h fasted [range 4·5-9·1]) but had no effect on overall exposure (difference in $AUC_{0\text{-}inf}$ between fed and fasted -0.013 [90% CI -0.11,0.08]). In part 3, rapid initial parasite clearance occurred in all participants following dosing (clearance half-life 6·6 h [95% CI 6·2-6·9] for 200 mg, 6·8 h [95% CI 6·5-7·1] for 300 mg, and 7·1 h [95% CI 6·6-7·6] for 900 mg). Recrudescence occurred in 4/5 (200 mg), 5/8 (300 mg), and 0/2 (900 mg) participants. Simulations performed using a pharmacokinetic/pharmacodynamic model predicted a single dose of 1100 mg would clear baseline parasitaemia by a factor of 10^9.

Interpretation

The safety, pharmacokinetic profile, and antimalarial activity of ZY-19489 in humans support its development as a novel antimalarial therapy.

Funding

Cadila Healthcare Ltd. and Medicines for Malaria Venture.

RESEARCH IN CONTEXT

Evidence before this study

New antimalarials with novel mechanisms of action are needed to combat the emergence of drug resistance and progress towards malaria elimination. The triaminopyrimidine compound ZY-19489 is a new antimalarial candidate. We searched PubMed up to May 25, 2021, using the terms “triaminopyrimidine” and “antimalarial”. A single publication outlining the preclinical development of ZY-19489 (previously known as “compound 12” and “AZ-13721412”) was reviewed. ZY-19489 was found to exhibit potent antimalarial activity towards asexual blood-stage *Plasmodium falciparum* parasites *in vitro* and in a mouse model of malaria. The compound did not exhibit appreciable activity towards the liver stage or sexual blood-stage of the parasite life cycle. The pharmacokinetic properties of ZY-19489 indicated the compound was amenable to use as a drug and appropriate safety margins were observed in toxicity studies in guinea pigs and rats. Together these findings supported the progression of ZY-19489 into clinical development.

Added value of this study

This is the first human clinical trial of ZY-19489. This trial integrates a single ascending dose (SAD) study, a pilot food effect study, and a volunteer infection study (VIS) using the *P. falciparum* induced blood-stage malaria model to characterise the safety, pharmacokinetics, and antimalarial activity of ZY-19489 in healthy adults. Our results demonstrate that ZY-19489 is well tolerated in humans up to the maximum dose tested of 1500 mg. ZY-19489 exhibited a moderate rate of oral absorption, with maximum plasma concentration occurring 5-9 h after dosing, and a moderate rate of elimination (half-life 50-97 h). Dosing following consumption of a high-fat meal delayed the rate of oral absorption compared to fasted dosing (maximum plasma concentration 12 h vs 6 h) but did not affect overall exposure. Single oral doses of ZY-19489 (200 mg, 300 mg, or 900 mg) resulted in rapid initial clearance of asexual

blood-stage parasitaemia (clearance half-life approximately 7 h). No evidence of *in vitro* drug resistance was found in recrudescent parasite populations.

Pharmacokinetic/pharmacodynamic analysis and dosing simulations predicted a single dose of 1100 mg ZY-19489 would clear baseline parasitaemia by a factor of 10^9.

Implications of all the available evidence

ZY-19489 is well tolerated in healthy males and females at doses that clear asexual blood-stage *P. falciparum*. Future studies to investigate dosing regimens and combination therapies of ZY-19489 with an appropriate partner drug in treating clinical malaria are warranted.

INTRODUCTION

The global burden of malaria remains high, with an estimated 229 million cases and 409,000 deaths in 2019.[1] The emergence and spread of artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong Subregion is a major threat to malaria control and elimination.[2] Novel antimalarial treatments are therefore required to combat drug resistance and reduce malaria morbidity and mortality.

The required properties of new antimalarial molecules have been outlined by defining a number of target candidate profiles.[3] These include molecules that clear asexual blood-stage parasitaemia rapidly to improve clinical symptoms, have activity against hypnozoites to prevent relapse (predominantly *P. vivax*), have activity against hepatic schizonts to offer chemoprotection, block transmission by targeting the sexual gametocyte stage, and block transmission by targeting the insect vector. Further, target product profiles have been defined for final antimalarial products based on their intended use.[3] For example, a treatment for acute uncomplicated *P. falciparum* malaria would require activity against asexual blood-stage parasites and gametocytes. An additional highly desirable property of such a treatment is single dose administration to avoid compliance issues associated with multiple dose regiments. It is likely that a combination of two or more compounds would be required to achieve these outcomes. Notwithstanding the above factors, antimalarial drug development faces a broad array of challenges associated with safety and tolerability considerations, use in pregnancy and paediatric populations, drug-drug interactions, food-effect, parasite drug resistance, formulation considerations, and cost of treatment.[3] These must all be considered in the decision to invest in the clinical development of novel antimalarials.

Triaminopyrimidines comprise a novel antimalarial class that was identified in a high-throughput screen against asexual blood-stage *P. falciparum* parasites.[4] Optimisation of the initial hit compound led to the development of ZY-19489 (previously referred to as "compound 12" and "AZ-13721412").[5] ZY-19489 (and its major active metabolite ZY-20486, previously referred to as "compound 9") displayed nanomolar potency towards asexual blood-stage *P. falciparum*, both *in vitro* and in a mouse model of malaria.[5] However, it lacked significant activity against liver stage or sexual forms of the parasite.[5] The mode of action of ZY-19489 is yet to be elucidated, although a novel mechanism is considered likely, since the compound retains activity against a panel of clinical isolates with a range of resistance patterns, as well as against laboratory strains with varying mechanisms of resistance to antimalarials currently in use or under development.[5] Together, the findings from preclinical studies[5] indicate that ZY-19489 is able to kill asexual blood-stage parasites rapidly with high potency, likely exhibits a novel mode of action, has a low propensity to select for the emergence of resistance, has a predicted pharmacokinetic profile in humans favourable for drug development, and is associated with monitorable toxicity, thus supporting the progression of the compound to clinical development as a component of a new treatment for acute uncomplicated *P. falciparum* malaria.

This report describes the first-in-human clinical trial aimed to characterise the safety, tolerability, pharmacokinetics, and antimalarial activity of ZY-19489 in healthy volunteers. The study was undertaken in three parts. The first part was a single ascending dose (SAD) study with oral administration of ZY-19489 or a matching placebo. The second part was a pilot food effect study with oral administration of ZY-19489, either in a fasted state or immediately following consumption of a high fat meal. The third part was a volunteer infection study (VIS) using the induced blood-stage malaria (IBSM) model[6] in which

malaria-naïve participants were inoculated with *P. falciparum*-infected erythrocytes and subsequently administered a single oral dose of ZY-19489. This work represents the first clinical investigation of a triaminopyrimidine antimalarial compound.

METHODS

Study design and participants

This study was conducted in three-parts. Part 1 was a first-in-human, double-blind, randomised, placebo-controlled, SAD study. Part 2 was an open label, two-period cross-over, randomised, pilot food effect study. Part 3 was an open-label VIS using the *P. falciparum* IBSM model.

Males and females (non-pregnant, non-lactating) of good health aged 18-55 years were eligible for inclusion; participants in the VIS were required to be malaria naïve (full eligibility criteria are listed in the supplementary file, page 17). The study was conducted at Nucleus Network (Brisbane, Australia) following approval by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee. All participants gave written informed consent before enrolment and received financial compensation for their time commitment. This study was registered on the Australian New Zealand Clinical Trials Registry (ANZCTR) with the trial reference identifiers ACTRN12619000127101 (Part 1), ACTRN12619001466134 (Part 2), and ACTRN12619001215112 (Part 3).

Randomisation and masking

In part 1, participants were randomised within each dose cohort to either ZY-19489 or placebo in a 3:1 ratio. Participants and investigators were masked to the identity of the treatment from the time of randomisation until database lock. Treatment identity was concealed by identical packaging and appearance of both ZY-19489 and placebo. An unmasked pharmacist allocated a randomisation number (generated using SAS®) to each participant as per the randomisation schedule immediately before dosing. In part 2, a single cohort of participants were randomised to receive initial ZY-19489 dosing in either a fasted

or fed condition in a 1:1 ratio, with randomisation performed as described above. No masking was performed. In part 3, all participants enrolled in cohort 1 received the same ZY-19489 dose, with no randomisation performed. Participants enrolled in cohort 2 were randomised to a dose group after malaria challenge and prior to ZY-19489 dosing. The randomisation schedule was generated using STATA 15. No masking was performed.

Procedures

The SAD was conducted in 6 dose cohorts (25, 75, 150, 450, 900, and 1500 mg). Following randomisation on day 0, participants received a single oral dose of ZY-19489 (Cadila Healthcare Ltd.) or placebo (microcrystalline cellulose, Dupont Nutrition Ireland) in capsule form with 240 mL water after fasting for at least 10 h; no food was allowed until 4 h after dosing. Dosing occurred under direct observation by clinic staff. A sentinel dosing strategy was employed for each dose cohort whereby two participants (one active and one placebo) were initially randomised and dosed. The investigator reviewed masked safety data up to at least 48 h after dosing before deciding to proceed with randomisation and dosing of the remaining six participants in the cohort. Participants were confined to the clinic for 72 h post-dosing and returned as outpatients for follow up visits until the end of study visit on day 28±3. The starting dose of ZY-19489 used in the SAD (25 mg) was calculated in accordance with guidance from the U.S. Food and Drug Administration.[7] Dose selection for each subsequent cohort was decided by the Safety Data Review Team (SDRT) based on pharmacokinetics, safety, and tolerability data from the previous cohort.

The pilot food effect study was conducted in a single dose cohort. Following randomisation on day 0, participants received a single oral dose of 300 mg ZY-19489 in either a fasted or fed condition. The fasted condition involved dosing after an overnight fast of at least 10 h.

The fed condition involved consuming a high-fat meal (approximately 150 calories from protein, 250 calories from carbohydrate, and 500-600 calories from fat) after an overnight fast of at least 10 h. ZY-19489 was administered 30 minutes after the start of the meal and participants were required to consume the whole meal prior to dosing. After a wash-out period of 28 days, participants crossed over to the opposite fed or fasted condition (period 2) and received a second 300 mg dose of ZY-19489. For both periods, dosing occurred under direct observation by clinic staff and participants were confined within the clinical unit for 72 h post-dosing and returned as outpatients for follow up visits. The end of study visit occurred on day 56±3. The dose of ZY-19489 used in part 2 was to be no more than a third of a dose that was determined to be well tolerated in part 1 to account for a possible increased exposure in a fed state.

The VIS was conducted in two cohorts with three dose levels of ZY-19489 (cohort 1: 300 mg; cohort 2: 200 mg or 900 mg). Participants were inoculated intravenously with *P. falciparum* 3D7-infected erythrocytes (~2800 viable parasites) on day 0. Parasitaemia was monitored on an outpatient basis up to twice daily until day 8 when ZY-19489 was administered as a single oral dose in the fasted state. Dosing occurred under direct observation by clinic staff. Participants were confined to the clinic for 72 h post-dosing and returned as outpatients for follow up visits until the end of study visit on day 36±3. Participants received a standard curative course of artemether-lumefantrine upon recrudescence, or on day 33±3 if recrudescence had not occurred. Recrudescence was defined as parasitaemia ≥5,000 parasites/mL with a 2-fold parasitaemia increase within 48 hours, or re-occurrence of malaria symptoms with a malaria clinical score >6. Recrudescent parasites were rescued into *in vitro* culture (prior to artemether-lumefantrine treatment) and resistance to ZY-19489 was determined (IC_{50} and IC_{90}; methodology described in the supplementary

file, page 10). Whole-genome sequence analysis of parasite DNA was also performed to investigate for selection of mutations that could confer resistance to ZY-19489 (supplementary file, page 9). The dose of ZY-19489 administered to the first cohort in the VIS (300 mg) was determined based on the safety, tolerability, and pharmacokinetic results obtained in part 1, and from human efficacious dose predictions from preclinical studies.[5] The dose was predicted to have a sub-curative effect (i.e. expected to be associated with recrudescence) to facilitate calculation of pharmacokinetic/pharmacodynamic (PK/PD) parameters. After review of safety, pharmacokinetic, and pharmacodynamic data, the SDRT decided that participants enrolled in cohort 2 would be randomised to receive 200 mg or 900 mg ZY-19489 to optimise definition of the exposure-response relationship.

ZY-19489 and ZY-20486 (the major active metabolite) concentration in plasma was determined using liquid chromatography tandem mass spectrometry (methodology described in supplementary file, page 13). Parasitaemia was measured using quantitative PCR (qPCR) targeting the gene encoding *P. falciparum* 18S rRNA[8]. Blood sampling time points for ZY-19489 concentration and parasitaemia measurements are specified in the supplementary file (page 21). Gametocytemia was measured in select participants at select time points after ZY-19489 dosing (based on whether 18S qPCR results suggested gametocytes may be present) using qRT-PCT targeting the female gametocyte specific transcript *pfs25*.[9]

Outcomes

The primary outcome of the study (all parts) was the incidence, severity, and relationship to ZY-19489 of adverse events (AEs). AEs were recorded from the time of first ZY-19489 dosing (parts 1 and 2), or inoculation with the malaria challenge agent (part 3), up to the end of the study. AE severity was recorded in accordance with the Common Terminology Criteria

for Adverse Events[10] (mild=grade 1; moderate=grade 2; severe=grade 3; life-threatening consequences=grade 4; death related to AE=grade 5). In addition, an AE was classified as a serious adverse event (SAE) if it met one of the following criteria: resulted in death, was life-threatening, required inpatient hospitalisation, resulted in persistent or significant disability, was a congenital anomaly, or was considered medically important. The investigator assessed whether AEs were related to ZY-19489, and to the malaria challenge agent in part 3 (unrelated, unlikely, possible, or probable). Safety assessments included clinical laboratory parameters, vital signs, physical examination, and 12-lead electrocardiographs (ECGs).

Secondary outcomes for all parts included non-compartmental PK parameters (for ZY-19489 and its major active metabolite ZY-20486); the effect of fed and fasted dosing on these parameters was a secondary outcome in part 2. Secondary outcomes in part 3 were the parasite reduction ratio over a 48 h period following dosing (PRR_{48}), the corresponding parasite clearance half-life ($PCt_{1/2}$), and the percentage of participants with recrudescent parasitaemia; and derived PK/PD modelling parameters (minimum inhibitory concentration [MIC], minimal parasiticidal concentration that achieves 90% of the maximum effect [MPC_{90}], and the estimated single dose to clear baseline parasitaemia by a factor of 10^6 and 10^9).

Exploratory outcomes included the effect of gender on pharmacokinetic parameters (part 1), the acquisition of resistance to ZY-19489 in recrudescent parasites (part 3), and the development of gametocytemia following ZY-19489 dosing (part 3). Other exploratory outcomes planned (drug concentration/QTc modelling from data in part 1, presence of unique metabolites of ZY-19489, infectivity of gametocytes to mosquitoes, *ex vivo* parasite viability) will be presented separately.

Statistical analysis

The target sample size in part 1 (8 participants per cohort randomised 3:1 ZY-19489:placebo) was based on general phase 1 trial experience and was considered appropriate to investigate for the primary safety outcome. Similarly, the intended sample size in part 2 (single cohort of 8 participants randomised 4:4 to dosing in the fasted-fed or fed-fasted sequence) was designed to provide preliminary information on the food effect associated with ZY-19489 dosing and was not based on formal statistical calculations. The intended sample size in the VIS (8 participants per cohort) was selected based on previous IBSM studies that successfully characterised the parasite clearance kinetics of various antimalarial compounds.[11-15]

Non-compartmental pharmacokinetic analysis was performed using Phoenix® WinNonlin® version 8·4 (Certara L.P., Princeton, New Jersey). A significant food effect in part 2 was considered to be excluded if the 90% confidence interval (CI) of the fed: fasting ratios fell within 80% to 125% for the geometric mean of log-transformed C_{max}, $AUC_{0\text{-}last}$ and $AUC_{0\text{-}inf}$.

Pharmacodynamic analyses of antimalarial activity were performed in R version 4·0·2. The PRR_{48} and parasite clearance half-life were estimated using the slope of the optimal fit for the log-linear relationship of the parasitaemia decay as described previously.[16]

PK/PD analyses were performed using non-linear mixed effects models. A population PK model was developed to obtain individual PK parameter estimates that adequately described the observed individual PK profiles. The PK/PD model was then built using the individual PK parameter estimates as regression parameters to estimate the relationship between ZY-

19489 plasma concentration and parasite killing. The MIC, MPC_{90}, and PRR_{48} were derived from the PK/PD model. Model evaluation and selection was guided by visual inspection of goodness of fit plots, of individual PK and PD profiles, plausibility and precision of parameter estimates, and fit statistics such as Bayesian information criterion. For each VIS participant, the individual dosing regimen required to clear baseline parasitaemia by a factor of 10^6 and 10^9 was predicted by simulations using the individual PK and PD parameters. The efficacious dose was then defined as the maximum individual predicted dosing regimen. Additional simulations were performed for paediatric dosing by assuming the same PK/PD relationship and scaling the individual PK parameters by allometry for relevant paediatric body weight. All data processing, PK and PK/PD modelling were conducted within R (v3.6.3) combined to the IQR package (v1.5.0) and MONOLIX (MLX2019R1). Further detail of PK/PD analysis methodology is included in the supplementary file (page 14).

Role of the funding source

Authors employed by Cadila Healthcare Ltd. (the study sponsor) and Medicines for Malaria Venture, who provided additional funding support, were involved in protocol development, study oversight, and data analysis and interpretation. All authors had access to primary data, accept responsibility for the accuracy and completeness of data, and were involved in the decision to submit for publication.

RESULTS

The study was conducted from 18 January 2019 to 24 August 2020 (dates for each cohort are listed in Table S12, supplementary file page 22). In total, 286 volunteers were screened for eligibility, with 215 excluded (Figure 1). A total of 71 participants were enrolled; 48 in part 1, 8 in part 2, and 15 in part 3.

The six ascending dose cohorts of part 1 (25 mg-1500 mg) each comprised 8 participants, with six randomised to ZY-19489 and two to placebo. The single cohort of part 2 comprised 8 participants, with four randomised to receive two doses of 300 mg ZY-19489 in a fasted-fed sequence, and four randomised to dosing in a fed-fasted sequence. The first cohort of part 3 comprised 8 participants, all dosed with 300 mg ZY-19489. Due to recruitment limitations, the second cohort was a split cohort comprised of 7 participants in total (cohort 2A [n=4] and 2B [n=3]). In cohort 2A, 2 participants were randomised to receive 200 mg and 2 participants were randomised to receive 900 mg ZY-19489. All 3 participants in cohort 2B were dosed with 200 mg ZY-19489.

All participants enrolled in part 1 and part 3 completed the study. One participant in part 2 was withdrawn from the trial due to an AE on day 27, the day prior to scheduled administration of the second ZY-19489 dose in the fed condition (AE described below). Available data from this participant were included in the analyses of study endpoints. There were similar proportions of males and females in each study part and dose group, and the majority of participants were Caucasian (Table 1).

In part 1, 26/36 participants (72%) dosed with ZY-19489 and 6/12 participants (50%) dosed with placebo experienced at least one AE (Table 2 and Table S1, supplementary file page 2).

AEs considered related to dosing were experienced by 18/36 participants (50%) dosed with ZY-19489 and 4/12 participants (33%) dosed with placebo (Table 2 and Table S2, supplementary file page 5). The incidence of drug-related AEs was highest in the 1500 mg dose group (6/6 participants) due to more frequent mild gastrointestinal symptoms following dosing (5/6 participants experienced nausea, and 4/6 participants diarrhoea, generally within a few hours of dosing). One participant vomited 4 hours after dosing with 75 mg ZY-19489 and another participant vomited 11 hours after doing with 900 mg ZY-19489; both events were considered possibly related to dosing. Headache was the most commonly reported AE overall, but no clear relationship with ZY-19489 dose was observed. Other than headache, nausea and diarrhoea, all other drug-related AEs were recorded for one or two participants in total. One severe headache was reported with onset 13 days after dosing with 900 mg in part 1, and was considered possibly related to ZY-19489 (6 h duration, resolved with ibuprofen). All other drug-related AEs were mild or moderate in severity.

In part 2, all 8 participants experienced at least one AE, the majority of which were of mild severity (Table 2 and Table S1, supplementary file page 2). AEs were considered to be related to ZY-19489 for 5/8 participants (63%) overall, with 3/7 participants (43%) and 4/8 participants (50%) experiencing at least one drug-related AE when dosed, fasted, and fed respectively (Table 2 and Table S2, supplementary file page 5). Headache was the most commonly reported drug-related AE, with 6 events reported in 3 participants overall (38%). All other drug-related AEs were reported for one participant only. There was one AE in part 2 that resulted in withdrawal of one participant from the study. The participant developed a grade 3 (severe) increase in aspartate aminotransferase (225 U/L, normal range 10-40 U/L) on day 27 (the day prior to scheduled dosing in period 2, fed state). The AE was considered to be unrelated to any study procedures, and likely due to a sudden increase in exercise.[17]

In part 3, all 15 participants experienced at least one AE of mild to moderate severity and frequently associated with malaria (Table 2 and Table S1, supplementary file page 2). As previously observed during the conduct of VIS studies using the IBSM model, these occurred at the time of clearance of parasitaemia after administration of the test antimalarial compound. Due to this chronology of events, it is often not possible to differentiate AEs related to malaria from those related to the test antimalarial (in this case AEs are recorded as related to both). The most commonly reported drug-related AE in part 3 was headache; other drug-related AEs reported for more than two participants in total were diarrhoea, nausea, and myalgia (Table 2 and Table S2, supplementary file page 5). One participant vomited 9 days after dosing with 300 mg ZY-19489 which was considered possibly related to dosing. There was one SAE in a participant who developed neutropenia with a neutrophil nadir of $0{\cdot}1\times10^9$/L (normal range $1{\cdot}5\text{-}6{\cdot}5\times10^9$/L) noted on day 8 following inoculation with *P. falciparum* and just prior to dosing with 300 mg ZY-19489. The participant subsequently developed a temperature of 38·1°C and was admitted to hospital and administered intravenous piperacillin/tazobactam. The participant's neutrophil count remained low until day 13, when a single subcutaneous dose of granulocyte colony stimulating factor was administered, with subsequent neutrophil recovery resulting in the participant being discharged the following day. The SAE was attributed to the malaria challenge since neutropenia is known to be associated with both naturally acquired[18] and experimentally induced[19] malaria. The neutropenia was unknown at the time of ZY-19489 dosing since clinical laboratory blood samples for cohort 1 were collected immediately prior to dosing on day 8, and results were not received in real time. The protocol was amended for cohort 2 to collect the clinical laboratory blood samples on day 7, thus enabling the results to be reviewed prior to dosing on day 8.

No clinically relevant changes in heart rate, blood pressure, respiratory rate, or body temperature were observed during the study with respect to ZY-19489 dosing. No participants had a post-dose QTcF value >470 msec upon ECG analysis. A QTcF change from baseline (pre-dose) >30 msec occurred in 5 participants (one participant dosed with 25 mg and one participant dosed with placebo in part 1, one participant dosed with 300 mg fasted in part 2, and two participants dosed with 300 mg in part 3). No participants had a QTcF change from baseline >60 msec.

Dose-related increases in ZY-19489 exposure were observed across the entire dose range (Figure 2A). Exposure (C_{max} and AUC) parameters were approximately dose-proportional at higher doses (450 mg, 900 mg, and 1500 mg), whereas at lower doses (25 mg, 75 mg, and 150 mg) exposure was sub-proportional (Table 3). ZY-19489 displayed a moderate rate of oral absorption (median t_{max} 5·0 h to 8·8 h across the dose range) and elimination (geometric mean $t_{1/2}$ 49·9 h to 97·0 h). Dosing with 300 mg ZY-19489 in the fed state resulted in a delayed rate of oral absorption compared to the fasted state (median t_{max} 12·0 h fed [range 7·5-16·0] vs 6·0 h fasted [range 4·5-9·1]) but no effect on overall exposure was apparent (Table 3 and Table S6, supplementary file page 7). Dose-related increases in exposure to the major metabolite of ZY-19489 (ZY-20486) were also observed across the entire dose range (Figure 2B). Exposure to ZY-20486 was 23-37% of exposure to the parent compound (Table S3, supplementary file page 6), while its elimination half-life was slightly longer than that of the parent compound (89.1 h to 122.8 h). Similar to the parent compound, the t_{max} of ZY-20486 was later in the fed state (48·2 h fed vs 24·1 h fasted), but no difference in overall exposure was observed (Table S6, supplementary file page 7). Females generally exhibited higher exposures (C_{max} & AUC parameters) to ZY-19489 and ZY-20486 compared to males

(Tables S4 and S5, supplementary file page 7), although this study was not powered to determine if the differences were significant. The PK profiles of ZY-19489 and ZY-20486 in participants in the VIS were comparable to those in the SAD study (Table 3).

A rapid reduction in parasitaemia occurred for all participants following ZY-19489 dosing in the VIS (Figure 3 A-C), with no apparent variation in the rate of parasite clearance across the dose groups. The rate of parasite clearance was similar between dose groups ($log_{10}PRR_{48}$ 2·21 [95% CI 2·09-2·33] for 200 mg; 2·13 [95% CI 2·03-2·23] for 300 mg; and 2·04 [95% CI 1·91-2·18] for 900 mg). The corresponding $PCt_{1/2}$ were 6·6 h (95% CI 6·2-6·9) for 200 mg, 6·8 h (95% CI 6·5-7·1) for 300 mg, and 7·1 h (95% CI 6·6-7·6) for 900 mg. Individual participant clearance parameters are presented in Table S7 (supplementary file page 8). Recrudescence occurred in 4/5 participants (80%) dosed with 200 mg ZY-19489 (8, 13, 15, and 16 days after dosing) and in 5/8 participants (63%) dosed with 300 mg ZY-19489 (8, 16, 19 [2 participants], and 22 days after dosing). Recrudescence was not observed in either of the 2 participants dosed with 900 mg up to 23 days post-dose. Gametocytemia was detected in participants after dosing with ZY-19489 (Table S8, supplementary file page 8). All participants were treated with artemether-lumefantrine and were confirmed to be aparasitaemic (using qPCR) by the end of the study.

Recrudescent parasites from 7 participants (n=5 for 300 mg and n=2 for 200 mg) were rescued into *in vitro* culture (prior to artemether-lumefantrine treatment) to screen for resistance to ZY-19489. IC_{50} and IC_{90} values were equivalent between all recrudescent parasite populations and the parental *P. falciparum* 3D7 strain (Figure S1, supplementary file page 11). Whole-genome sequence analysis revealed no single nucleotide polymorphisms in any coding sequences or any copy number variations in the genomes of any of the

458 recrudescent parasite populations, compared with the parental strain (Table S9,
459 supplementary file page 12).
460
461 Population PK modelling indicated that the PK profile of ZY-19489 was adequately
462 described by a two-compartment model, with linear elimination, zero-order absorption, lag
463 time and combined residual error (Table S10, supplementary file page 15). The relationship
464 between ZY-19489 plasma concentrations and parasite killing was best described by an E_{max}
465 model (Table S11, supplementary file page 16). The estimated median (range) MIC was 8·4
466 ng/mL (1·2-16·8) and MPC_{90} was 39·3 ng/mL (3·8-85·8). Simulations of an adult patient
467 population indicated that a single dose of 420 mg and 1100 mg would clear baseline
468 parasitaemia by a factor of 10^6 and 10^9 respectively. In paediatric patients with scaling of the
469 individual PK parameters to body weights of 10, 15 and 25 kg by allometry, single doses of
470 230 mg, 240 mg and 250 mg respectively were predicted to clear baseline parasitaemia by a
471 factor of 10^6; while single doses of 1040 mg, 950 mg and 910 mg respectively were predicted
472 to clear baseline parasitaemia by a factor of 10^9. The inverse relationship between body
473 weight and dose predicted to clear parasites by a factor of 10^9 can be explained by the effect
474 of body weight on clearance and volume of distribution using an allometric function.
475

DISCUSSION

This study represents the first clinical investigation of the safety, pharmacokinetics, and antimalarial activity of the novel triaminopyrimidine ZY-19489. Combining a single ascending dose, pilot food effect, and volunteer infection study into the first in human study has enabled the rapid accrual of data to expedite clinical development. Safety results indicated that ZY-19489 is well tolerated when administered to healthy participants as a single oral dose up to 1500 mg. The incidence of drug-related AEs, namely mild gastrointestinal symptoms (nausea and diarrhoea), was higher in the 1500 mg dose group compared to lower dose groups or placebo. AEs considered related to ZY-19489 were transient in nature and all but one were mild or moderate in severity. It will be important to closely monitor gastrointestinal adverse events in future clinical trials involving larger sample sizes.

The favourable pharmacokinetic profile of ZY-19489 observed in healthy volunteers confirms the potential for it to be developed as a new antimalarial treatment. A moderate rate of oral absorption following dosing in a fasted state (maximum plasma concentration 5-9 h post-dose) was slowed when dosed immediately after a high fat meal (maximum plasma concentration 12 h post-dose) but had no effect on overall exposure and therefore no expected clinically relevant impact on safety and efficacy. ZY-19489 exhibited a moderate rate of elimination ($t_{1/2}$ 50-97 h), which was longer than predicted from preclinical data (36 h).[5] As preclinical studies have indicated that ZY-19489 is a major substrate of cytochrome P450 3A4 (CYP3A4), future studies will be needed to explore drug interaction with CYP3A4 inducers and inhibitors.

Results obtained in the VIS indicate that ZY-19489 has potent activity against blood-stage *P. falciparum*, with single oral doses of 200, 300, or 900 mg resulting in a parasite clearance half-life ($PCt_{1/2}$) of 6·6 h to 7·1 h. Although this is slower compared to fast acting artemisinin derivatives such as artesunate[20] ($PCt_{1/2}$ 3·2 h [95% CI 3·0-3·3]), it is similar to that of mefloquine[13] ($PCt_{1/2}$ 6·2 h [95% CI 5·7-6·7]). Doses of 200 mg or 300 mg ZY-19489 were insufficient to prevent recrudescence in all participants, whereas neither of the two participants administered 900 mg developed recurrent parasitaemia up to 23 days post-dose. It is important to note that the VIS was not designed to characterise a curative dose experimentally; rather it was aimed to estimate a curative dose using population PK/PD modelling and dose simulations. These analyses indicated that a single dose of 1100 mg ZY-19489 would be sufficient to clear baseline parasitaemia by a factor of 10^9 in adults, while a dose of 910 to 1040 mg would clear an equivalent parasite burden in children with a body weight ranging from 10 to 25 kg. Although the safety and tolerability profile of ZY-19489 was confirmed in healthy adults up to a single dose of 1500 mg, studies in children will be required to determine its suitability in paediatric populations. Further, although estimation of the single efficacious dose of ZY-19489 was a predefined outcome of the current study, future investigations into multiple-dosing regimens will likely be considered in future clinical development. Such investigations will also need to take into account potential partner drugs to be used with ZY-19489 in a new antimalarial combination treatment. The PK/PD data obtained in the current study will be important in informing these clinical development considerations.

No evidence of acquisition of drug resistance was detected *in vitro* in any of the recrudescent parasite populations examined, suggesting that recrudescence was due to insufficient drug exposure rather than resistance. Whole-genome sequence analysis revealed no coding

sequence single nucleotide polymorphisms or gene amplifications in any recrudescent parasite populations. The absence of development of drug resistance in this trial supports the results of preclinical studies that observed a very low frequency of spontaneous resistance under *in vitro* drug selection with triaminopyrimidines (<1 in 10^{10} asexual blood stage parasites).[5] Nevertheless, it will be important to monitor for the development of drug resistance in future clinical trials.

Although this trial provides valuable first-in-human data on ZY-19489, it has limitations. The population examined were healthy adult males and females (18-55 years of age), the majority of whom self-selected their race as Caucasian. It will be important to investigate potential differences in safety and pharmacokinetics in target patient populations. Additionally, although the pharmacokinetic data obtained in this trial indicated that females exhibited somewhat higher exposures compared to males, the study was not powered to determine whether these differences were significant. Further investigation of gender differences in pharmacokinetics in future trials with larger sample sizes are warranted. Finally, although VIS have been shown to predict the activity of investigational antimalarials in studies in endemic populations,[12,13,21,22] further study of the pharmacodynamic effect of ZY-19489 in such populations will be required to confirm that the selected dose is sufficient to effect cure.

In conclusion, this first-in-human study of the novel triaminopyrimidine ZY-19489 supports its further clinical development as a new antimalarial. ZY-19489 is well tolerated in healthy adults at doses that clear blood-stage *P. falciparum* and there is no clinically relevant effect of food on its pharmacokinetic profile after oral administration. Combination treatments are the focus of new antimalarial therapies to reduce the risk of drug resistance development and ensure clearance of different parasite lifecycle stages. This will be important for ZY-19489

given the apparent absence of activity against the liver stage and transmissible sexual stage of the parasite lifecycle observed in preclinical studies.

Contributors

BB (principal investigator), JSM and SW (associate investigators), and MF (project manager) were responsible for the acquisition of data and contributed to study design, analysis and interpretation of data. KK (sponsor project director) and DP (sponsor medical director) were responsible for overall study design and directing trial activities. HBP was responsible for overall management of the study activities and study performance. SS was responsible for statistical analysis and results interpretation. HP, MJ, and AG were responsible for drug concentration measurements and pharmacokinetic analyses and contributed to overall trial design and interpretation of data. SC was responsible for overseeing safety aspects of the trial. IDR and JJM were responsible for aligning clinical trial design with overall project strategy and providing input in results analysis and interpretation. SL performed the analysis of ZY-19489 antimalarial activity, assisted with VIS study design and data interpretation. DAF designed and led the experiments to investigate ZY-19489 resistance in recrudescent parasites, with the research conducted by ID, TY and SM. CB, AR, and AF were responsible for performing the pharmacokinetic/pharmacodynamic analysis and interpreting the results.

The trial sponsor (Cadila Healthcare Ltd.) designed the study with input from all authors. All authors contributed to data interpretation and reviewed the manuscript. BB, HBP and KK accessed and verified the data. A professional medical writer employed by QIMR Berghofer Medical Research Institute, and funded by Cadila Healthcare Ltd., drafted the manuscript.

Declaration of interests

HBP, HP, SS, DP, MJ, AG, and KK are employed by Cadila Healthcare Ltd., the study sponsor. SC, JJM, CB, and IDR are employed by Medicines for Malaria Venture (MMV) who provided funding support for this trial. BB (principal investigator) and JSM (associate investigator) received funding from Cadila Healthcare Ltd. and MMV to perform the study. All other authors declare no competing interests.

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Data sharing

Individual participant data that underlie the results reported in this article will be made available after de-identification (text, tables, figures, and appendices). The study protocol and statistical analysis plan will also be made available. Data and related documents will be available immediately following publication and ending 5 years following article publication to researchers who provide a methodologically sound proposal. Proposals should be directed

to the corresponding author (kevinkumarkansagra@zyduscadila.com). To gain access, data requestors will be required to sign a data access agreement.

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TABLES

Table 1. Demographic profile of participants

		Single ascending dose study							Food effect study		Volunteer infection study		
		Placebo N=12	**25 mg N=6**	**75 mg N=6**	**150 mg N=6**	**450 mg N=6**	**900 mg N=6**	**1500 mg N=6**	**300 mg fed-fasted N=4**	**300 mg fasted-fed N=4**	**200 mg N=5**	**300 mg N=8**	**900 mg N=2**
Age [years]	Mean ± SD	31·0 ± 11·7	25·8 ± 6·5	23·8 ± 5·5	28·8 ± 4·9	27·5 ± 8·1	26·7 ± 5·6	26·0 ± 6·2	25·5 ± 6·0	27·5 ± 13·2	26·6 ± 10·1	28·5 ± 8·0	28·5 ± 10·6
Sex [n (%)]	Male	6 (50·0)	4 (66·7)	3 (50·0)	5 (83·3)	2 (33·3)	3 (50·0)	4 (66·7)	0	2 (50·0)	3 (60·0)	5 (62·5)	1 (50·0)
	Female	6 (50·0)	2 (33·3)	3 (50·0)	1 (16·7)	4 (66·7)	3 (50·0)	2 (33·3)	4 (100)	2 (50·0)	2 (40·0)	3 (37·5)	1 (50·0)
Race [n (%)]	Caucasian	10 (83·3)	5 (83·3)	5 (83·3)	5 (83·3)	5 (83·3)	6 (100)	5 (83·3)	3 (75·0)	3 (75·0)	5 (100)	6 (75·0)	2 (100)
	Asian	2 (16·7)	0	1 (16·7)	1 (16·7)	0	0	1 (16·7)	1 (25·0)	0	0	0	0
	Mestizo	0	0	0	0	1 (16·7)	0	0	0	0	0	0	0
	Native Hawaiian/ Other Pacific Islander	0	0	0	0	0	0	0	0	0	0	1 (12·5)	0
	Other	0	1 (16·7)	0	0	0	0	0	0	1 (25·0)	0	1 (12·5)	0
BMI [kg/m^2]	Mean ± SD	24·8 ± 4·1	23·3 ± 2·2	23·7 ± 2·7	21·8 ± 2·3	22·9 ± 3·3	23·5 ± 1·7	22·2 ± 2·8	26·4 ± 1·7	23·7 ± 3·8	21·1 ± 1·3	29·2 ± 2·1	28·0 ± 2·5
Height [cm]	Mean ± SD	172·8 ± 10·6	180·5 ± 8·7	172·0 ± 10·9	176·7 ± 6·8	177·2 ± 7·5	175·2 ± 12·3	174·0 ± 10·9	167·5 ± 4·7	171·8 ± 5·7	171·0 ± 6·7	173·0 ± 11·1	173·5 ± 14·8
Weight [kg]	Mean ± SD	75·0 ± 18·0	76·4 ± 12·5	70·2 ± 9·3	68·6 ± 10·6	72·3 ± 12·8	72·6 ± 10·8	67·3 ± 10·9	74·3 ± 8·8	70·5 ± 15·0	62·1 ± 7·8	87·6 ± 10·3	85·4 ± 22·1

BMI: body mass index; SD: standard deviation; ND: not determined.

Table 2. Adverse events summary

AE category	Single ascending dose study							Food effect study		Volunteer infection study[c]		
	Placebo N=12	25 mg N=6	75 mg N=6	150 mg N=6	450 mg N=6	900 mg N=6	1500 mg N=6	300 mg Fed N=7	300 mg Fasted N=8	200 mg N=5	300 mg N=8	900 mg N=2
	Number of participants with an AE (%); number of AEs											
Any AE	6 (50·0); 13	3 (50·0); 7	4 (66·7); 6	4 (66·7); 8	5 (83·3); 9	4 (66·7); 12	6 (100); 22	5 (71·4); 12	7 (87·5); 13	5 (100); 33	8 (100); 66	2 (100); 9
Any AE related to ZY-19489/placebo	4 (33·3); 5	1 (16·7); 1	2 (33·3); 4	3 (50·0); 4	2 (33·3); 3	4 (66·7); 9	6 (100); 18	3 (42·9); 5	4 (50·0); 7	4 (80·0); 10	7 (87·5); 28	2 (100); 6
Serious AE[a]	0	0	0	0	0	0	0	0	0	0	1 (12·5); 1[d]	0
AE resulting in discontinuation	0	0	0	0	0	0	0	0	1 (12·5); 1[e]	0	0	0
Grade 2 AE[b]	1 (8·3); 1	0	1 (16·7); 1	0	0	2 (33.3); 5	1 (16·7); 1	2 (28·6); 2	1 (12·5); 1	6 (75·0); 17	7 (87·5); 15	2 (100); 2
Grade 2 AE related to ZY-19489/placebo	1 (8·3); 1	0	1 (16·7); 1	0	0	1 (16·7); 4	0	1 (12·5); 1	1 (12·5); 1	2 (40·0); 5	6 (75·0); 9	1 (50·0); 1
Grade 3 AE[b]	0	0	0	0	1 (16·7); 1[f]	1 (16·7); 1[g]	0	0	1 (12·5); 1[e]	0	0	0
Grade 4 AE[b]	0	0	0	0	0	0	0	0	0	0	1 (12·5); 1[d]	0

AE: adverse event. [a]A serious adverse event was one that fulfilled at least one of the following criteria: resulted in death, was life-threatening, required hospitalisation, resulted in a persistent or significant disability, was a congenital anomaly, was considered medically important. [b]The medical assessment of adverse event severity was recorded in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) v5.0, published 27 November 2017 (mild=grade 1; moderate=grade 2; severe=grade 3; potentially life-threatening=grade 4; death related to AE= grade 5). [c]Adverse events were recorded from administration of the malaria challenge agent in the volunteer infection study. [d]The grade 4 adverse event was decreased neutrophil count occurring prior to dosing with ZY-19489. This event also met the criteria for a serious adverse event because it resulted in hospitalisation. The event was considered related to the malaria challenge agent. [e]The adverse event leading to discontinuation was an increase in aspartate aminotransferase (grade 3) occurring the day prior to the scheduled second ZY-19489 dose in the fed state. The participant was withdrawn from the

678 study without administering the second dose. The event was considered unrelated to ZY-19489. [f]The grade 3 AE was severe abdominal pain which was considered unrelated to

679 ZY-19489. [g]The grade 3 AE was severe headache which was considered related to ZY-19489.

Table 3. Plasma ZY-19489 pharmacokinetic parameters

	Single ascending dose study						Food effect study		Volunteer infection study		
	25 mg N=6	**75 mg N=6**	**150 mg N=6**	**450 mg N=6**	**900 mg N=6**	**1500 mg N=6**	**300 mg Fed N=7[a]**	**300 mg Fasted N=8**	**200 mg N=5**	**300 mg N=8**	**900 mg N=2**
C_{max} (ng/mL)	6·5 (24·4)	27·2 (59·5)	48·8 (52·3)	435·3 (38·6)	1039·4 (24·2)	1871·4 (30·5)	152·0 (38·1)	188·8 (42·6)	124·1 (37·6)	180·7 (32·9)	1044·4 (106·7)
t_{max} (h)	7·00 (5·50, 9·00)	7·01 (4·50, 24·01)	8·50 (5·00, 24·03)	5·00 (4·50, 6·50)	5·00 (4·50, 10·13)	8·75 (5·00, 10·15)	12·00 (7·50, 16·00)	6·00 (4·50, 9·05)	5·50 (4·50, 10·00)	5·75 (4·06, 7·50)	3·75 (3·00, 4·50)
$AUC_{0\text{-}last}$ (h*ng/mL)	341·6 (70·8)	2147·8 (37·1)	2272·3 (80·8)	23790·8 (47·9)	50309·1 (49·1)	74520·3 (22·2)	14130·6 (41·1)	14397·4 (40·7)	6815·6 (43·7)	13518·8 (52·6)	36227·1 (171·1)
$AUC_{0\text{-}inf}$ (h*ng/mL)	486·2 (59·8)	2338·9 (34·2)	2398·8 (78·6)	24253·9 (49·4)	50927·2 (49·5)	74851·1 (22·3)	14501·1 (41·0)	14692·4 (40·5)	7031·9 (41·7)	13903·7 (50·8)	37276·2 (163·8)
$t_{1/2}$ (h)	76·6 (36·9)	81·5 (27·5)	49·9 (43·6)	97·0 (62·6)	80·5 (35·9)	87·1 (20·0)	95·8 (19·6)	86·6 (14·2)	67·9 (34·8)	86·0 (48·4)	59·2 (70·1)
CL/F (L/h)	0·054 (54·5)	0·032 (31·8)	0·064 (77·8)	0·018 (52·9)	0·016 (59·2)	0·019 (36·7)	0·021 (49·5)	0·020 (38·4)	0·026 (42·2)	0·022 (47·7)	0·022 (162·8)
Vz/F (L)	5·685 (29·9)	3·771 (25·7)	4·499 (43·5)	2·599 (26·3)	2·051 (32·0)	2·519 (20·9)	2·857 (33·0)	2·551 (33·1)	2·788 (27·6)	2·679 (31·1)	2·065 (54·4)
λ	0·009 (37·8)	0·009 (25·5)	0·014 (42·5)	0·007 (63·8)	0·009 (36·5)	0·008 (18·2)	0·007 (20·0)	0·008 (16·3)	0·010 (33·2)	0·008 (48·4)	0·011 (75·0)

Data are geometric means (coefficient of variation [%]) except t_{max} which is median (minimum, maximum). [a]One participant was not dosed in the fed state due to the occurrence of an adverse event the day prior to scheduled dosing. C_{max}: maximum observed concentration; t_{max}: time to reach the maximum observed concentration; $AUC_{0\text{-}last}$: area under the concentration-time curve from time 0 (dosing) to the last sampling time at which the concentration is at or above the lower limit of quantification; $AUC_{0\text{-}inf}$: area under the concentration-time curve from time 0 (dosing) extrapolated to infinity; $t_{½}$: apparent terminal half-life; CL/F: apparent total clearance; Vz/F: apparent volume of distribution; λ: apparent terminal elimination rate constant.

FIGURES

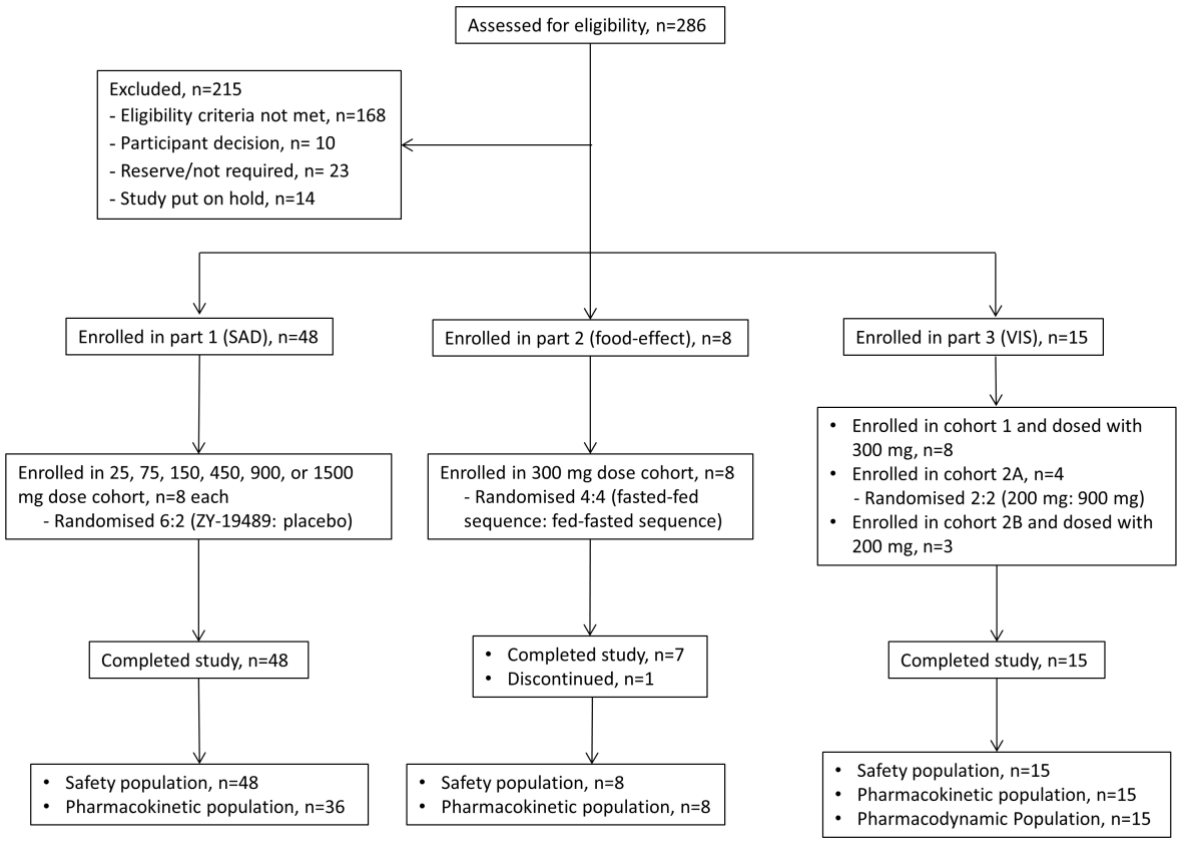

Figure 1: Trial profile. In part 1, single ascending doses (SAD) of ZY-19489 (25–1500 mg) or placebo were tested in six cohorts. In part 2, 300 mg ZY-19489 was administered to a single cohort of participants fasted and following consumption of a high fat meal (food effect). Part 3 was a volunteer infection study (VIS) consisting of three dose groups (200, 300, 900 mg ZY19489); participants were dosed 8 days following challenge with blood-stage *P. falciparum*. Parts 2 and 3 started after documentation of safety and pharmacokinetics data of the first five cohorts (up to the 900 mg dose cohort) in part 1.

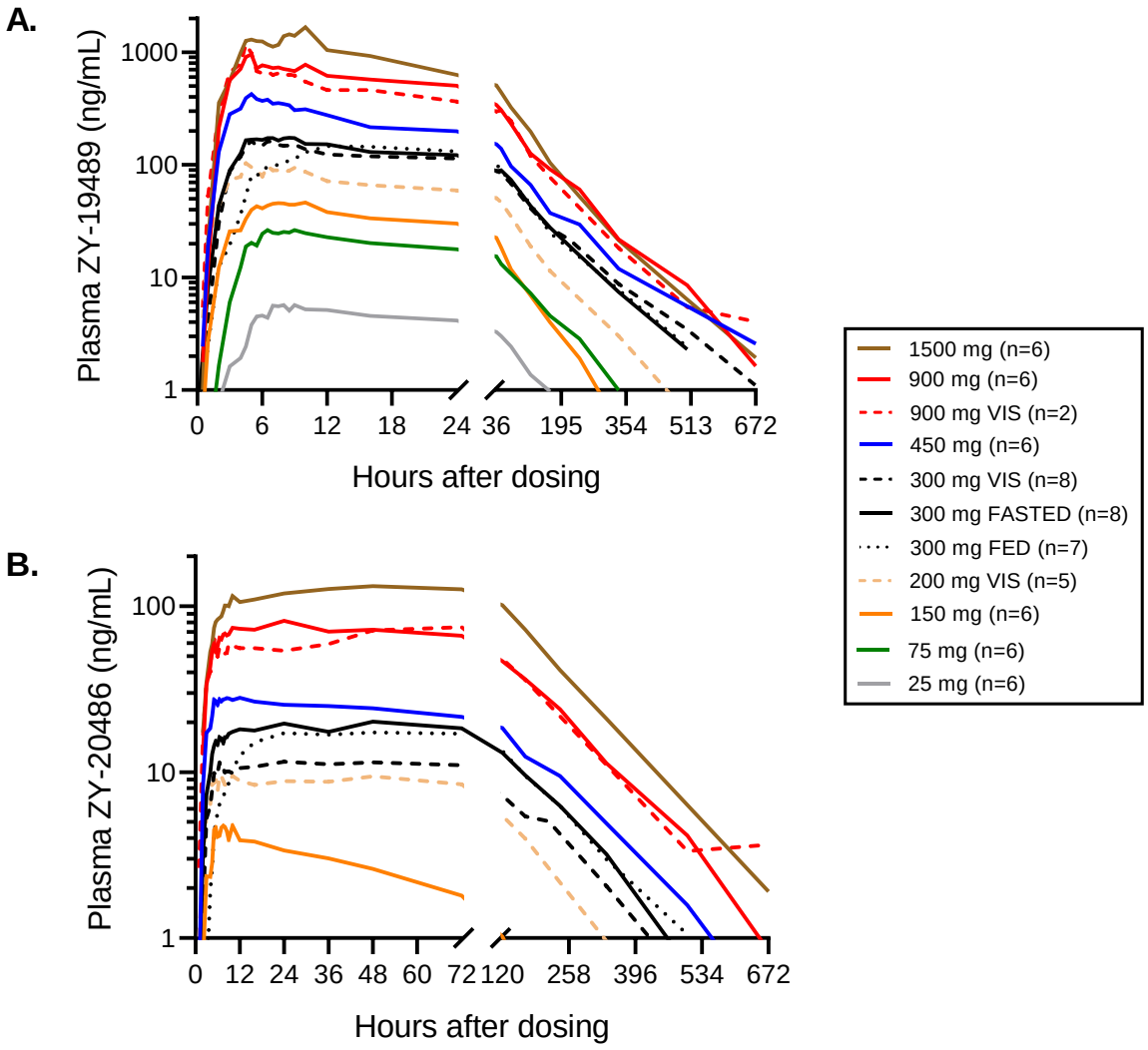

Figure 2: ZY-19489 and ZY-20486 (major active metabolite) plasma concentration-time profiles. Plots represent the mean of the ZY-19489 (A) or ZY-20486 (B) plasma concentration of each dose group over the study. Plasma concentrations were measured using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). For the purpose of graphing on a $\log_{10}$ logarithmic scale, time points at which ZY-19489 or ZY-20486 could not be detected were substituted with a value of 1 ng/mL (lower limit of quantitation was 1 ng/mL for ZY-19489 and 2 ng/mL for ZY-20486). ZY-20486 plots for the 25 mg and 75 mg dose groups are not presented because mean concentrations were below the lower limit of quantification at all time points.

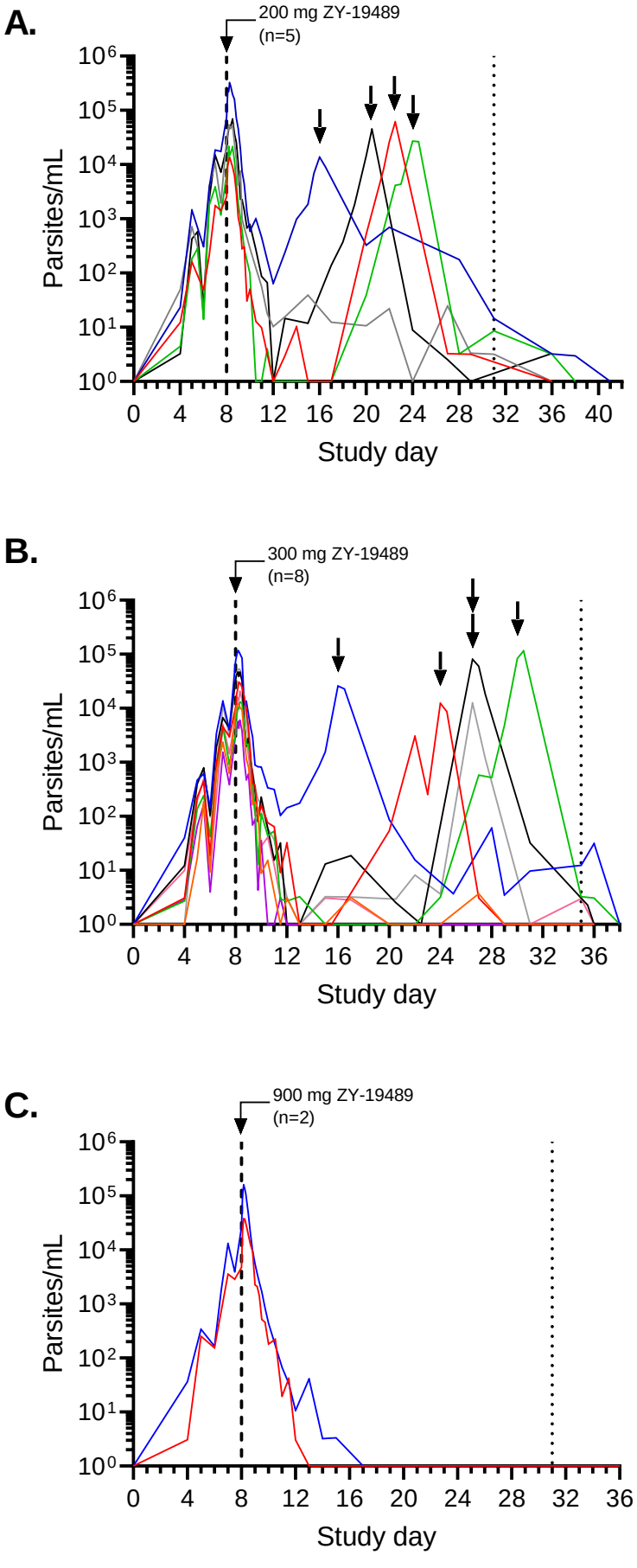
A.
200 mg ZY-19489
(n=5)
Parsites/mL
Study day
B.
300 mg ZY-19489
(n=8)
Parsites/mL
Study day
C.
900 mg ZY-19489
(n=2)
Parsites/mL
Study day

Figure 3: Individual participant parasitaemia-time profiles in the volunteer infection study. Participants were inoculated intravenously with *P. falciparum*-infected erythrocytes and were administered a single oral dose of 200 mg (A), 300 mg (B), or 900 mg (C) ZY-19489 after 8 days (indicated by the vertical dashed line). Parasitaemia was monitored using qPCR targeting the gene encoding *P. falciparum*18S rRNA. Artemether-lumefantrine was administered in response to recrudescence of parasitaemia (indicated by the vertical arrows) or 25±3 days after ZY-19489 dosing if recrudescence was not observed (indicated by the vertical dotted line). For the purpose of graphing on a $\log_{10}$ logarithmic scale, time points at which parasitaemia could not be detected were substituted with a value of 1 parasite/mL.

SUPPLEMENTARY MATERIAL

Supplementary_methods_and_results.pdf